INHERITANCE IN THE ASEXUAL REPRODUCTION OF CENTROPYXIS ACULEATA.

by

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Dissertation submitted to the Board of University Studies of the Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy.

Baltimore, June, 1917.

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Inheritance in the Asexual Reproduction of Centropyxis aculeata.

Introduction.

The "Pure Line" Concept. Since Johannsen (1903), as a result of his studies on self-fertilized plants, first enunciated the concept of "pure lines", the phenomenon to which he gave this name has been shown, by a multitude of other investigators, to be of very general occurrence. It has been proved that the same general state of affairs exists, not only in self-fertilized plants, but also in plants reproducing vegetatively, by tubers or cuttings, and in animals reproducing by parthenogenesis, budding, and fission. It is unnecessary to enter into the details of these investigations. I will only mention the papers of East (1910) on the potato, of Agar (1914) and Ewing (1914, 1914a, 1916) on daphnids and aphids reproducing parthenogenetically, of Hanel (1908) and Lashley (1915, 1916) on Hydra, and of Barber (1907), Jennings (1908) and Winslow and Walker (1909) on protozoans and bacteria. All these investigators entirely corroborated the results and conclusions of Johannsen.

And what are these conclusions? Simply that in organisms reproducing asexually and in homozygous organisms reproducing by self-fertilization, each species is not an ultimate unit, but is itself made up of a large number of

diverse strains, called "pure lines", "clones", or "biotypes", differing from each other, but each one constant and invariable in its own hereditary constitution. This last idea, that every individual of any given clone is precisely the same in its genetic make-up as any other individual of the same clone, is the basic idea of the pure line concept. As a corollary, it follows that all the variations found within a single clone are purely somatic and not heritable. In this case, it is evident, selection working within a clone could have no effect. The testing of this hypothesis, then, is of the greatest importance for any general consideration of the mechanism of evolution.

This problem has already attracted the attention of numerous investigators. Some have completely confirmed the conclusions of Johannsen. The more important of these papers have already been cited. But there are others who have maintained that hereditary variations do occur within clones, and that selection within a clone may have an effect.

Pearson (1910), subjecting the data of the earlier pure line workers to a more accurate statistical analysis, found evidence indicating that such inheritance might exist. Stout (1915), Stocking (1915), Middleton (1915), and Jennings (1916) have all succeeded in isolating, by selection, hereditarily diverse strains within single clones. The present paper adds another similar case to this list.

Problems. Accepting, as we must, the fact that there do exist, within a single species, diverse strains or races, there are two main problems which claim our attention.

The main problem, undoubtedly, is the one which has already been discussed. Are "pure lines" really pure ? Do hereditary variations occur within the clone ? It is this problem which is attacked in the present paper.

A second problem, intimately connected with the first, is presented by the very existence of diverse strains. How did these diverse strains originate ? Or, to bring the problem within the horizon of the experimentalist, can new strains arise from old ones, and if so, how ? The number of diverse strains which exist in nature is almost beyond belief. In my own work with Centropyxis, out of over thirty individuals, isolated from a single pipette-ful of debris, taken from a single dead lotus stem, there were no two which, after a month's multiplication and careful study, could be surely assigned to the same clone. Where did these diverse races come from ? How long have they been in existence ? How do new races arise from existing races ? To these questions we have, as yet, no answer.

Plan of the present investigation. The object of my work with Centropyxis was to find out whether heritable variations do or do not occur within a single clone. Since many investigators have reported that such variations are not to be found, it may be taken for granted that it will be no easy task to find them, even if they do occur. It is not sufficient, then, simply to pick a character at random, and determine whether its variations are or are not hereditary. One must first show that diverse clones exist in the given species, and that these clones differ from each other in certain inherited characters. Then these characters may be

studied, and those which are most favorable for showing the inheritance of variations within the clone, if it exists, may be determined. When this has been done, and not until then, one is in a position to put the question to a fair test.

There are two ways in which the inheritance of variations within the clone may be determined. The first is a statistical method, making use of the coefficient of correlation. The inheritance of diversities within the clone is always accompanied, unless some other factor intervenes, by a significant positive correlation between parents and their offspring. Also, as Pearson (1910) has pointed out, if the size of the coefficient of correlation diminishes as we go back to more and more remote ancestral generations, it indicates that inheritance of variations occurs within single clones. This criterion of a "diminishing ancestral correlation" applies to coefficients of correlation computed either from a single clone or from a population containing many clones.

All the more recent investigators have realized, however, that results expressed solely as coefficients of correlation are often inconclusive and hard to interpret. In particular, if an experiment extends over a considerable space of time, a gradual change in environmental conditions which affect the character studied will simulate a true inheritance of variations in the most perfect manner, so far as the resulting coefficients of correlation are concerned.

The real test for the ocurrence of heritable variations within a clone must be the success or failure of an attempt to isolate, by selection, hereditarily diverse groups within

a single clone.

In my own work on Centropyxis, both of these methods have been employed, and the evidence drawn from coefficients of correlation was used as a guide in the subsequent selection experiments.

In closing this brief introduction to the problem, I desire to express my deep indebtedness to Dr. H. S. Jennings, who suggested this problem to me, and who has greatly assisted both the experimental work and the preparation of this paper by his kindly advice and criticism.

Material - Centropyxis aculeata.

Descriptive. The lobose rhizopod, Centropyxis aculeata Stein, is an excellent subject for genetic investigation. It is closely related to Difflugia, the subject of Jenning's recent (1916) paper and, like Difflugia, its protoplasm is surrounded by a spine-bearing shell which fulfils all the requirements for genetic study. That is, it presents definitely measureable structural characters which are heritable, yet very variable, and which are entirely unaffected by growth or environmental influences during the life of the individual. The shape of the shell is even more favorable for study than that of Difflugia. The individuals used in this study correspond closely to the description of variety discoides Penard (1902). In this form the chitinous shell, studded with sand grains and diatom tests, is decidedly flattened (see Figure 2). The upper surface is convex and the lower slightly concave. Seen from above, as in Figure 1, the outline of the shell is nearly circular, and the thin "anterior" edge, with its smooth outline, is clearly distin-

Figure 1.

Dorsal view of living individual of Centropyxis aculeata.

X 450.

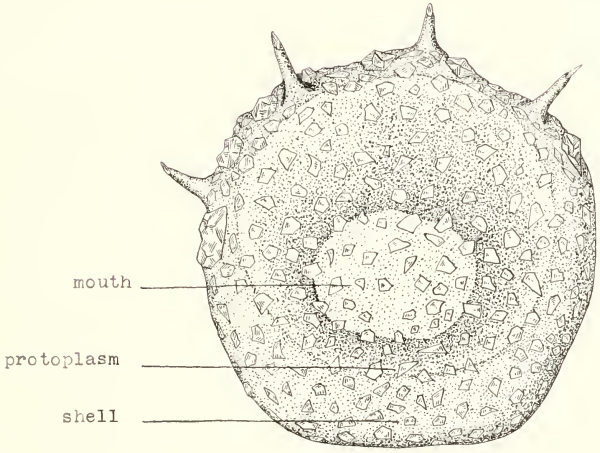
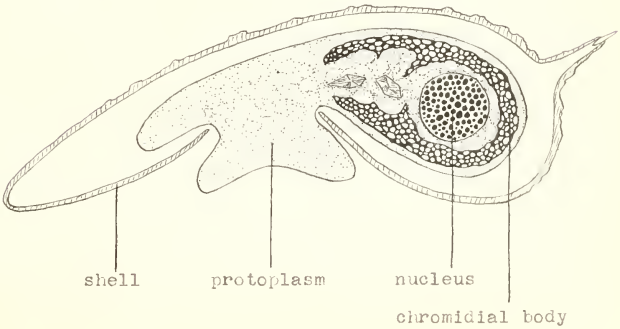


Figure 2.

Sagittal section of a specimen of Centropyxis aculeata.
X 500.



guishable from the thick, rough "posterior" edge, which may bear from one to eleven spines, each tipped by a grain of quartz which looks like the cork in a bottle. In all but the oldest shells, the outline of the mouth, which is situated at the top of a funnel-like infolding of the lower surface, is plainly visible through the shell, even when the organism is viewed from the upper side.

In order to permanently record the characters of an individual, then, it was necessary only to transfer the living organism to a minute drop of water on a slide and to trace the outline of shell and spines with a camera lucida, under the high power of the compound microscope. By focussing down a little the outline of the mouth was then added. Such camera outlines were made of every individual used in the statistical part of the work, and in one of the selection experiments. Some of them are reproduced, reduced in size, as illustrations for the present paper.

The chitinous shell surrounds an ameboid protoplast. At the time of cell division the protoplast swells, probably by the absorption of water, and projects out of the mouth of the old shell. This projection assumes the size and shape of a new shell, but one with its "antero-posterior" axis in just the opposite direction to that of the old shell. To the surface of this mold flow various sand-grains and diatom tests which have been picked up by the parent organism and stored within its body. Now, over the entire surface of the exposed protoplast, a chitinous sub-

stance is secreted, in which, as it hardens, the foreign particles become firmly embedded. After the new shell is formed, the parent protoplast is partially retracted into the old shell, the nucleus divides, and ultimately the protoplast pulls apart into two organisms, and the parent and its offspring separate.

The regular mitotic division of the nucleus during this process has been described and figured by Schaudinn (1903, 1911). Unfortunately, although a chromidial body is present, (see Figure 2), no account of its behavior during cell division has been published.

The new shell, when first formed, is almost white, in striking contrast to the dark brown color of old shells. The darker coloration is taken on gradually, first appearing as a faint yellowish tinge, which slowly deepens to brown. Even when four or five individuals have come from one in the course of a week or so, their relative ages, and therefore their parentage, is unmistakably indicated by their color. In practise, however, it was only very rarely that either of the two products of a division had time to divide again before they were separated. At ordinary laboratory temperatures, during the cooler months, Centropyxis divides about twice a week, at most. Inspection of the cultures at intervals of two or even three days was ordinarily sufficient to insure the isolation of parent and progeny before either had divided a second time.

In closing this brief description of the organism used in this experiment, I wish again to emphasize the fact that

the new shell is completely produced by the parent organism before nuclear division begins. It is simply handed on to one of the products of the ensuing fission. Further, the new shell is in no sense molded on the old one. This is particularly evident in Centropyxis, since, as figure 3 shows, the axes of old and new shells lie in exactly opposite directions. The number of spines and the shape of the shell, then, must be determined by precisely the same kind of determiners that underlie all the other hereditary characters of the species.

Previous breeding work on Centropyxis. The only other investigator who has studied the reproduction of Centropyxis aculeata is Schaudinn (1903, 1911). In his account of the life history of this species, he includes a few notes on the vegetative reproduction. Most of these statements I have been unable to confirm. He states, for instance, that the new shell is always larger than the old one. This I find not to be true. The new shell may be either slightly larger or slightly smaller than the old one. In line 30 (to be described later), with over 750 recorded fissions, the average size index of the parents was 21,969 sq.microns, that of the offspring 21,624 sq.microns. Schaudinn also says that he was never able to get an individual to divide more than once, and concludes that after this single fission the old individual lives on for some time and firally dies. In my cultures, individuals have repeatedly divided as many as ten times, some as many as fifteen times.

I have made no experiments on the sexual reproduction of Centropyxis, and will not discuss the life cycle. It is briefly described in Schaudinn's paper (1903). I can

Figure 3.

Position of old and new shells in the division of Centropyxis.

Lateral view.

only state that my cultures have repeatedly been interrupted, usually in January or February, by what I take to be epidemics of sexual reproduction. At the beginning of such epidemics, the protoplasts of nearly every individual in the cultures divide into a number of small amebae, which show no trace of a formed nucleus, but contain scattered chromatinic granules. These amebae escape from the shell, leaving it empty. I was unable to follow their further history.

Methods.

Cultural technique. In keeping Centropyxis in cultures in the laboratory, my aim was to keep the environmental conditions as near like the natural ones as was possible. Each individual was isolated in the depression of a hollow-ground slide, in about five drops of pond water. To this water was added, at the time of isolation, a few strands of the Oedogonium, in the mats of which this rhizopod was living, as well as some debris taken from among the matted filaments of the alga. This debris furnished diatoms, the principal constituent of the food of the rhizopod, as well as sand grains to be used in the construction of new shells.

In these miniature aquaria the water was changed every two or three days, and once a week all the individuals were transferred to freshly prepared slides, to avoid any possibility of an injurious accumulation of either excretion products or bacteria. Whenever a fission occurred, the new individual was removed with a capillary

pipette, outlined with the camera lucida, and transferred to a new culture slide.

Records were kept on small sheets of paper, each of which contained the number of a given individual, the date upon which it was isolated, its camera outline, and the numbers and dates of isolation of all of its progeny. Later, during the process of working up the data, the spine number and certain measurements of the outline drawing were added. Since these drawings, made from the living organism while the new shell was still clean and translucent, gave all the data required, no attempt was made to preserve the shells of individuals when they died or were discarded.

In some of the later work, when attention was centred wholly on the inheritance of spine number, the records were much less elaborate, consisting merely of the number of the specimen, the date on which it was isolated, its spine number, and the corresponding data for all of its offspring.

Statistical methods and use of terms. The following measurements were taken on each of the camera outlines. Diameter of shell and mouth in the "antero-posterior" axis. Diameter of shell and mouth in an axis at right angles to the "antero-posterior" axis. To secure a number proportional to the area of the shell, the two diameters were multiplied together. This was called the "shell size index". In order to obtain a number related in some way to the form of the shell, the "antero-posterior" diameter was divided by the other diameter. This was called the "shell form ratio".

The "mouth size index" and "mouth form ratio" were computed in the same way. All measurements were made in microns, giving the actual dimensions of the shell.

The coefficients of correlation given in this paper were computed by the improved methods devised by Dr. Jennings (see especially Jennings 1916, p.416). So many of these constants are given that it seems inadvisable to attempt to publish even all of the correlation tables, much less the extensive pedigrees on which they are based. A few of the more important tables are included in the appendix.

A word as to terminology. In this paper, following the usage of Shull (1912) and Johannsen (1913), the term "pure line" will be considered as restricted to homozygous organisms reproducing by self-fertilization. The various strains of Centropyxis considered in this paper are properly to be spoken of as "clones", that is, families arising from a single ancestor by asexual reproduction. The more general terms "strains", "lines" and "races" are considered as synonymous and as having no specific meaning.

Inheritance within a population.

Existence of diverse clones. The first requisite, in any study of inheritance within the asexual progeny of a single individual, is to be sure that the diverse clones, whose existence we assume in our population, really exist there. Next one must demonstrate that there exist between different clones hereditary diversities in the characters which are to be studied. Only too often (in the re-

cent papers of Ewing (1914, 1914a, 1916), for example) this precaution has been neglected.

My first cultures of Centropyxis aculeata began in October, 1914, with the isolation of fifty individuals from a pipetteful of debris taken from a mat of Oedogonium growing on a lotus stem in the Homewood pond, Baltimore. About twenty of these lines died out during the first month, wwhile I was learning the best culture methods. At the end of this time the thirty remaining lines all seemed to be distinct from each other. To keep the cultures within manageable bounds, only the four largest clones were continued for two months more. In considering inheritance within a population, then, the population consists only of these four clones, numbers 9, 30, 41 and 43, containing in all 1049 individuals.

It was evident, from the very first, that at least two distinct strains existed in my material. A glance at Table I or Figure 4 shows clearly that line 30 differs most decidedly from the other three lines. Their small size, rapid fission rate and high spine number differentiate individuals of this line from the others at first sight.

The other three lines resemble each other more closely. The differences between them are more minute. Line 43 is the most clearly distinct of the three. Its higher fission rate alone would suffice to separate it from the others, and its distinctness is further shown by the slightly smaller size and lower spine number of its members.

The other two lines, 9 and 41, are closely allied. The most striking difference between them lies in the higher

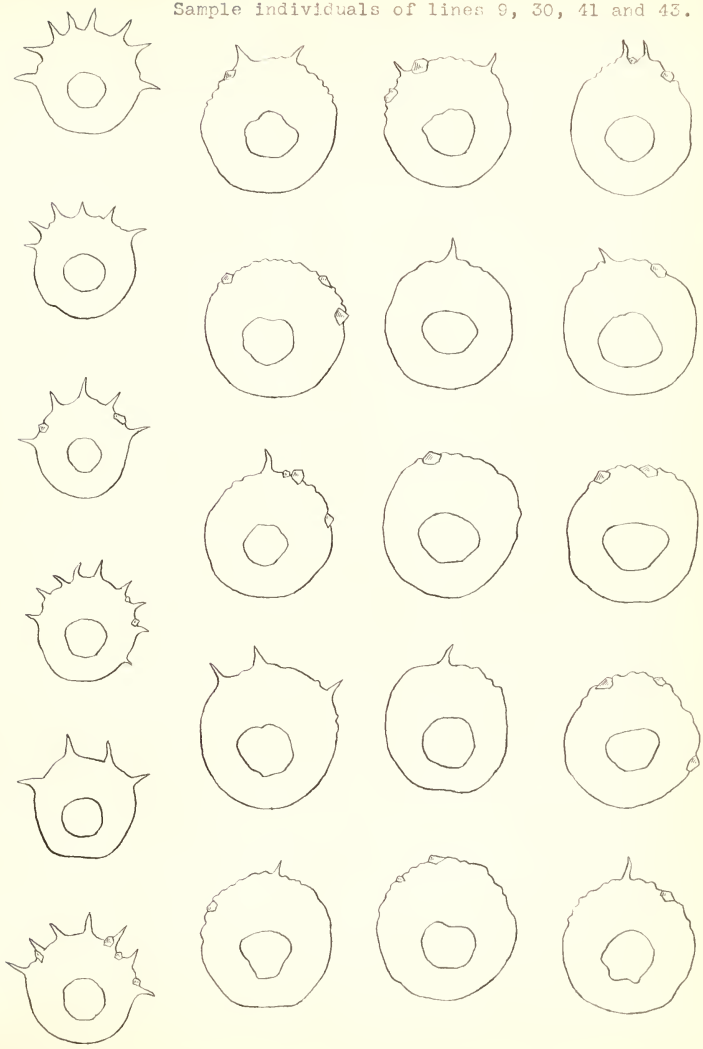
Table I.

Characters of the diverse lines.

Character	Line 9.	Line 30.	Line 41.	Line 43.
No.of individuals .	93	749	126	81
Av. length.	197.17	149.80	196.32	194.06
Av. breadth.	188.25	144.50	188.33	184.02
Av. size index.	371.25	216.48	370.19	357.23
Av. form ratio.	1.048	1.038	1.044	1.056
Av. mouth size index.	45.76	25.75	46.04	40.06
Av. mouth form ratio.	.926	.920	.879	.923
Av. spine number.	1.161	6.271	1.40	1.662
Av. number of days between two fissions.	11.53	6.12	11.31	9.40

Figure 4.

Sample individuals of lines 9, 30, 41 and 43.



Line 30. Line 41. Line 43. Line 9.

average spine number of line 41, shown in Table I and Figure 5. Shell size and form are almost identical, as is also fission rate. The size and form of the mouth show slight differences. To anticipate the conclusions of this paper, it seems not improbable that lines 9 and 41 are to be derived ultimately from a common ancestor.

It is to be noted, in connection with Table I, that the characters of size, spine number and fission rate show large differences between diverse clones, while the differences in form of shell and mouth are small.

When one recalls that the original ancestors of these lines were isolated at the same time and from the same place, and that the individuals of the different clones were cultivated at the same time, under the same conditions, the slides containing them intermingled in the same moist chambers, it seems unnecessary to adduce any further evidence to prove that diverse strains, differing in all the characters studied, do exist in Centropyxis.

Ancestral correlations within a population. In order to have a basis for comparison for the coefficients of correlation showing the inheritance of diversities within the single clones, coefficients of correlation were computed to show the degree of such inheritance within the population. In Table II are given the parental and grandparental coefficients of correlation for all the characters studied. In this table two points stand out clearly.

First, the correlation is very high for shell size and high for spine number and mouth size, but low for the two

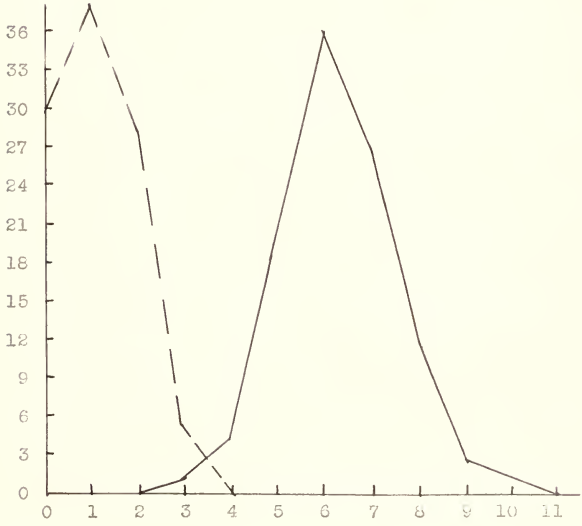
Table II.

Coefficients of correlation for the population.

Character.	Parental correlation. (1040 ind.)	Grandpar. correlation. (997 ind.)
Spine number.	.806 ± .007	.785 ± .008
Shell size.	.903 ± .004	.773 ± .008
Shell form.	.107 ± .022	.019 ± .021
Mouth size.	.732 ± .010	.677 ± .011
Mouth form.	.142 ± .021	.098 ± .021

Figure 5.

Graphs showing the percentage distribution of spine numbers in lines 9, 30, 41 and 43.



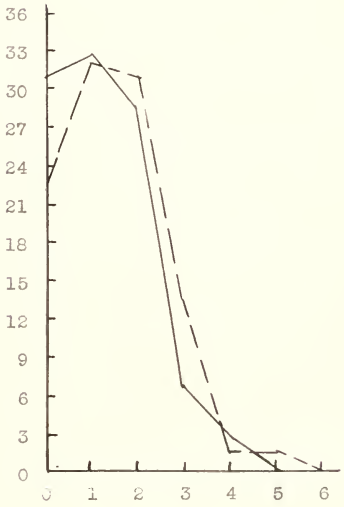
Lines 43 and 30. The ordinates are percentages, the abscissae numbers of spines.

Line 30- full line.

Line 43- broken line.

Figure 5.

Graphs showing the percentage distribution of spine numbers in lines 9, 30, 41 and 43.



Lines 9 and 41. The ordinates are percentages, the abscissae numbers of spines.

Line 9- full line.

Line 41- broken line.

form characters. This agrees with the conditions found in Table I. There the distinctions between the clones were definite in size and spine characters, but indefinite in form characters. So already two of the characters chosen for study have been practically eliminated for future work. It is perhaps significant that these characters are ratios, a kind of character with which much biometrical work has been concerned.

In the second place, it is noteworthy that, in every case, the grandparental coefficient is decidedly smaller than the parental. As Pearson (1910) has pointed out, according to the pure line hypothesis the offspring should resemble any other individual of the same clone, for example, their grandparent, just as much as they do their parent. Pearson's main argument against the evidence of the earlier pure line workers is this phenomenon of the diminishing ancestral correlation, which he considers satisfactory evidence that inheritance of variations within the pure line is occurring.

Inheritance within single clones.

Measured by the coefficient of correlation.

Ancestral correlations in line 30. Because of its rapid rate of division, many more individuals of line 30 were obtained than of any of the other races. The fact that this single clone contains 749 individuals makes it much the most suitable for statistical study. The other clones, lines 9, 41 and 43, with 93, 126 and 81 individuals, respectively, will be useful only for comparison. The

numbers concerned are too small to admit of definite conclusions from these three lines.

In Table III are given the coefficients of correlation for the first (parental), second (grandparental), third and fourth ancestral generations of line 30. It is at once evident that the five characters considered fall into two groups.

The parental coefficients for spine number and shell size are significant, in comparison with their probable errors. All the coefficients for these two characters are positive, and there is a regular decrease in their size as one passes from more recent to more remote ancestral generations. This evidence points toward the inheritance of variations in size and spine number, even within the single clone.

The other three characters, shell form, mouth size and mouth form, show no such evidence of inheritance. The parental coefficients are less than three times their probable errors, many of the other coefficients are negative, and no diminishing ancestral correlation is to be found. It can be said only that while variations in these characters may possibly be inherited, this table gives no evidence leading to such a conclusion.

Ancestral correlations in the other clones. In Table IV are given, for comparison, the coefficients of correlation for the first and second ancestral generations in lines 9, 41 and 43.

In all of these lines, the numbers available were so

Table III.

Coefficients of correlation for line 30.

Character.	Ancestral generations- 1. (755 ind.)	2. (754 ind.)	3. (744 ind.)	4. (693 ind.)
Spine number.	.682 ± .024	.034 ±.024	.009±.025	.007±.026
Shell size.	.253 ± .023	.026 ±.025	.012±.025	.019±.026
Shell form.	.062 ± .025	-.045 ±.025	-.056±.025	-.011±.026
Mouth size.	.024 ± .024	.013 ±.025	-.033±.026	-.056±.026
Mouth form.	.066 ± .024	-.043 ±.025	.066±.026	-.008±.026

small that no definite conclusions one way or the other can be drawn from them. As a glance at the table will show, they give no evidence for the inheritance of variations within the clone. Many of the coefficients, in both generations, are negative, hardly any are large enough to be significant, and in the few cases where there is a diminishing ancestral correlation, it is probably due to chance alone. One thing, only, is worth noting. The coefficients for spine number, in both generations, are all positive. This fact has no significance by itself, but, in comparison with the conditions shown by all the other characters, it may be considered to support the conclusion that variations in spine number may be inherited within the clone.

To sum up the purely biometrical part of the work, we may say-

1. Diverse clones exist in Centropyxis.
2. Within a population, diversities in size of shell and mouth, and in spine number are decidedly inherited, while diversities in form of shell and mouth are only slightly inherited.
3. Within single clones, the evidence from coefficients of correlation indicates that variations in shell size and spine number are inherited. The evidence that variations in the other characters are inherited is weak or lacking.

It was now decided to choose a single character for intensive study of the effect of selection. The statistical work has elimirated the two form characters, as showing only slight correlation even in a population.

Table IV.

Coefficients of correlation for lines 9, 41 and 43.

Line 9.

Character.	Parental correlation. (93 ind.)	Grandpar. correlation. (81 ind.)
Spine number	.148 ± .069	.041 ± .075
Shell size.	-.121 ± .069	-.060 ± .075
Shell form.	-.135 ± .069	.120 ± .074
Mouth size.	-.071 ± .069	-.155 ± .073
Mouth form.	-.053 ± .070	-.252 ± .070

Line 41.

Character.	Parental correlation. (130 ind.)	Grandpar. correlation. (116 ind.)
Spine number.	.006 ± .060	.125 ± .060
Shell size.	-.062 ± .060	-.074 ± .063
Shell form.	-.057 ± .060	.152 ± .062
Mouth size.	-.017 ± .060	.117 ± .059
Mouth form.	.185 ± .058	-.061 ± .060

Line 43.

Character.	Parental correlation. (81 ind.)	Grandpar. correlation. (69 ind.)
Spine number.	.144 ± .073	.215 ± .073
Shell size.	.088 ± .075	-.019 ± .080
Shell form.	-.082 ± .075	-.053 ± .080
Mouth size.	-.148 ± .074	.117 ± .079
Mouth form.	-.080 ± .075	.240 ± .075

There remain only spine number and the two size characters, which must be considered together, since they are closely correlated.

Shell size, as has been shown, gives the highest parental coefficient of correlation, both in the population and in line 30. In the other lines the coefficient was often negative. Spine number shows a decided correlation in the population and a significant one in line 30, and in all the other lines the coefficient was always positive. It is easy to imagine a way in which shell size could be "inherited" in a purely mechanical sense, since the old shell acts as a limit to the possible growth of the organism within it, and this organism then serves as a mold over the surface of which the new shell is secreted. Finally, spine number is the character which appears to have the least relation to the physiological activity of the organism, and is the one which can be most accurately and easily determined. For these reasons, spine number was chosen as the subject for selection experiments.

After the objections which have just been raised to the use of shell size as a subject for selection experiments, it is advisable to determine whether variations in shell size are correlated with variations in spine number. This is especially necessary, since in our population we find that the large lines have few spines and the small lines many spines. Within the clone, however, this does not hold. The coefficient of correlation between shell size and spine number for the 745 individuals of line 30 is .019 ± .025, that is, for all practical purposes, zero.

Inheritance proved to occur by selection experiments.

Selection experiments 1 and 2 - Contradictory results of two brief experiments in mass selection. In order to bring out clearly the reasons which led me to abandon mass selection for individual selection based on a progeny test, it seems worth while to give a brief account of two experiments in mass selection which came to an abrupt and unexpected end. The first of these experiments was ended by an epidemic of sexual reproduction, the second by a period of extremely hot weather which probably acted indirectly by accelerating the multiplication of undesirable bacteria.

Neither of these experiments lasted long enough for the application of the real test of their effectiveness, the free multiplication of two altered strains in comparison with each other. But since selection consists only in allowing certain individuals to breed and denying that privilege to others, a good idea of whether selection is being effective can be gained by comparing the average spine number of the progeny produced by high- and low-selected groups during the same period. Such comparisons for selection experiments 1 and 2 are given in Tables V and VI.

Selection experiment 1. Table V shows the progressive effect of selection in a small many-spined clone closely resembling line 30. The progenitor of this race was isolated from algae taken from the Homewood pond on October 8,1914. On November 9, 52 individuals had been secured by the multiplication of the original one, and selection

Table V.

Selection experiment 1, mass selection.

High series- parents with 6 or more spines.

Low series- parents with 5 or less spines.

Mean of clone (52 ind.) when selection began- 6.56 spines.

Between selections.	Progeny produced in High series. ind.	mean sp.no.	Low series. ind.	mean sp.no.	Difference between High and Low means.
1 and 2	49	6.57±.10	47	6.35±.10	.22±.14
2 and 3	30	7.36±.12	23	5.75±.14	1.61±.18
3 and 4	9	7.76±.22	7	5.50±.25	2.26±.32

began. On December 14 an epidemic of sexual reproduction put an end to the experiment.

In this experiment simple mass selection was used. The clone was divided into two parts, the individuals with six or more spines being classed as high, those with five or less as low. In each group only those progeny whose spine numbers lay on the same side of the mean (6.56 spines) as their parent's were allowed to reproduce.

As Table V shows, the difference in mean spine number between the progeny produced by the high and low groups was only .22 of a spine after the first selection, but rose to 1.61 spines after the second, and to 2.36 spines after the third. Apparently, then, in this clone, three selections sufficed to isolate within the clone high and low strains with a mean spine number of about 7.75 and 5.5 spines, respectively. This quick and decided effect of selection was most unexpected, and it is most unfortunate that the onset of sexual reproduction ended this experiment so prematurely. The numbers involved are too small to allow of any definite conclusion from an experiment of such short duration.

Selection experiment 2. In Table VI is shown the course of a similar experiment in a clone with fewer spines and of larger size. The progenitor of this clone was isolated on July 3, 1915, from among algae attached to a dead stick in an inlet of Lobska pond near Woods Hole, Mass. Selection was started on July 28, when the clone contained 55 individuals. The last individual of this

Table VI.

Selection experiment 2, mass selection.

High series- parents with 2 or more spines.

Low series- parents with 1 or 0 spines.

Mean of clone (55 ind.) when selection began- 1.51 spines.

Between selections.	Progeny produced in High series. ind.	mean sp.no.	Low series. ind.	mean sp.no.	Difference between high and low means.
1 and 2	25	1.96±.14	29	0.31±.13	1.65±.19
2 and 3	5	1.40±.30	9	0.00	1.40±.30
3 and 4			5	0.40±.30	

race died on August 16, after a period of very hot weather.

When selection began, the mean spine number of the clone was 1.51 spines. The high and low groups therefore consisted of individuals with two or more and one or no spines, respectively. The method of selection was the same as in experiment 1. Although the clone died out before more than one or two selections could be made in most of the lines, Table VI shows that this clone behaved very differently from the one used in experiment 1. The first selection gave a decided difference (1.65 spines) between high and low groups, as against a very slight one in the first experiment (.22 spines). And after the first selection no further result was noticeable. This differs decidedly from the steady progressive effect which was found in experiment 1.

In both these experiments mass selection was used. In fact, in almost all the experiments on the effect of selection within pure lines or clones, mass selection exclusively has been used. Mass selection is based entirely on the external appearance of the individual, and it is evident that the degree of effectiveness of the selection depends very largely on the extent to which the genetic constitution of the individual resembles its external aspect. Formerly mass selection within populations was extensively practiced, and one of the greatest achievements of the pure line workers has been the pointing out that by selecting on a basis of the character of the progeny produced by an individual, the same results can be

obtained with much fewer selections. This is true, of course, because the average character of the progeny of an individual gives a much more accurate test of its hereditary constitution than does its external appearance.

If this is true in a population, may it not also be true within a clone ? And if one seeks for an effect of selection within a clone, why not use the type of selection which has been found to be most effective in populations ?

Selection experiment 3. Quick effect of individual selection based on progeny. In accordance with the train of reasoning set forth above, in my next experiment individual selection, based on the average spine number of the progeny, was used. On October 10, 1915, an individual was isolated from the Homewood pond. After it had produced five progeny it was discarded. Each of these progeny, in turn, produced five offspring and was then discarded. Of these five sets of offspring, the set with the highest average spine number and the set with the lowest were allowed to produce five progeny each. The other three sets were discarded. Then another selection was made, the set of progeny with the highest average spine number being retained on the high side, and vice versa on the low side. This method of selection was continued for another generation.

Table VII shows the pedigree of this experiment. In this table, 5a, 5b and 5c are the progeny of individual 5; 5a1, 5a3, 5a4 and 5a5 are the progeny of 5a; and so on. The numbers in parentheses, following the numbers which

Table VII.

Selection experiment 3. Individual selection, based on the character of the progeny.

Generation 1. 5 (1.0)

Generation 2. 5a (0.8) 5b (0.3) 5c (0.2)

Generation 3.
High series. 5a1 (1.5) 5a3 (0.0) 5a4 (1.0) 5a5 (0.0)

Low series. 5c1 (0.3) 5c2 (0.2) 5c5 (1.0)

Generation 4.
High series. 5a1a (1.2) 5a1c (0.0) 5a1d (1.0)

Low series. 5c2a (0.2) 5c2b (1.0) 5c2c (0.6) 5c2d (0.3)

Generation 5.
High series. 5a1a1(1.8) 5a1a2(1.7) 5a1a4(1.0) 5a1a5(1.2)

Low series. 5c2a2(0.2) 5c2a3(0.4) 5c2a5(0.2)

Generation 6 and following generations.

High series-56 individuals derived from 5a1a1-
average spine number--- 1.68 ± .09

Low series-51 individuals derived from 5c2a2-
average spine number--- 0.22 ± .002

Difference between high and low means---- 1.46 ± .085

represent the individuals, are the average spine numbers of their five (in a few cases, six) progeny. All individuals which did not produce at least five progeny have been omitted from the table.

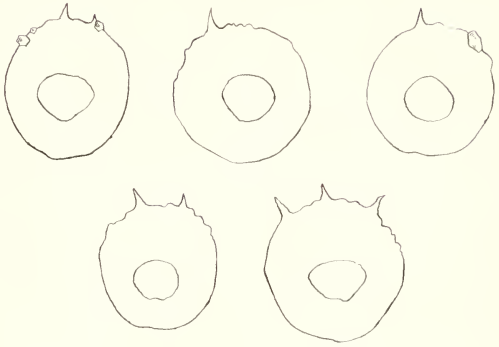
On December 14, after four selections had been made in both high and low groups, selection was discontinued, and the final individuals 5a1a1 and 5c2a2 and their progeny were allowed to multiply freely. Because of cold weather, the fission rate was very low, and when about fifty individuals had been produced in each group the experiment was ended on February 3, 1916.

The numbers concerned in this experiment are not large, but the difference between the means of the two final groups (1.46 spines) is so great in proportion to its probable error (.085) that there can be no doubt that in this case selection has isolated, within a single clone, two strains with decidedly different average spine numbers. In Figure 6 are shown a few characteristic members of the two groups, from camera outlines.

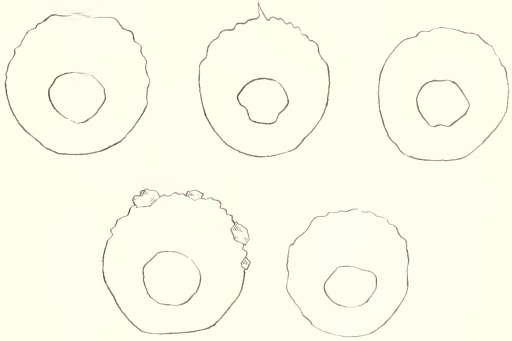
In this experiment there is one possibility of error. When one is selecting individuals instead of masses of individuals, a single line instead of a number of lines, an excellent opportunity is offered for the multiplication of any mutation that may occur. Recognizing this fact, and being now quite convinced in my own mind that selection within the clone was having an effect, I turned to mass selection once more. In mass selection, with many parallel lines, there is no chance for the extreme multiplication of a mutation, and if one does occur it changes the

Figure 6.

Sample individuals of high and low groups at end of experiment 3.



High series.



Low series.

final result only to a very slight degree. And if selection within a clone is really effective, mass selection on a large scale, in a favorable race should show such an effect. (By a favorable race, I mean, of course, one in which the external appearance of an individual is a fairly accurate index of its genetic constitution).

Selection experiment 4. Decided effect of mass selection in a favorable race. On June 29, 1916, an individual of Centropyxis was isolated from material collected from a freshwater pond on Cuttyhunk Island near Woods Hole, Mass., and was allowed to multiply. The course of this experiment is summarized in Table VIII. On August 1, when the line comprised 133 individuals, it was divided into two groups. This race was of large size, with a medium number of spines. On August 1 the average spine number was almost exactly 2.5 spines. All individuals with 0, 1 or 2 spines, 63 in number, were set aside as progenitors of low lines, and all individuals with 3or 4 spines (there were 70 of these) became progenitors of high lines. As soon as any of these individuals divided, the new one was examined. In the low lines, if the new individual had 3 or 4 spines, it was destroyed; but if it had 0, 1 or 2 spines, it was retained and its parent destroyed. In the high lines, of course, the reverse procedure was followed. Such selection was continued until August 26, only a few days before I had to leave Woods Hole. From August 26 to September 3, inclusive, no selection was practiced, and all the remaining individuals of both groups were allowed to

Table VIII.

Selection experiment 4, mass selection.

High series-parents with 3 or 4 spines.

Low series-parents with 0, 1 or 2 spines.

Period of	Time.	no.of ind.	av. sp. no.	Difference between high and low lines.
Multiplication.	June 29-July 8.	9	3.11 ± .22	
	July 9-18.	28	2.39 ± .14	
	July 19-30.	96	2.44 ± .08	
Selection.	Aug.1-10.			
	High lines.	146	2.71 ± .05	
	Low lines.	133	2.16 ± .07	.55 ± .09
	Aug. 11-20.			
	High lines.	167	2.55 ± .05	
	Low lines.	146	2.07 ± .07	.48 ± .09
	Aug. 21-25.			
	High lines.	89	2.77 ± .06	
	Low lines.	79	1.77 ± .07	1.00 ± .09
Multiplication after Selection	Aug. 26- Sept. 3.			
	High lines.	192	2.75 ± .05	
	Low lines.	180	1.76 ± .05	.99 ± .069

multiply freely.

During this time more than 175 offspring were produced by each group. The average spine numbers of these two sets of progeny differ by nearly a whole spine, with a probable error so small as to be negligible. This experiment, I think, proves conclusively that the effect of selection which has been demonstrated in experiment 3 is due to the inheritance of minute variations in spine number within the clone.

In this mass selection experiment, the appearance of a mutation has little effect on the ultimate result. Something comparable to a mutation did, in fact, appear in the low series of experiment 4. During the first period of multiplication, individual ldl, with one spine, began producing progeny all of which had no spines. Progeny with no spines were comparatively rare among the other low lines. All these progeny of ldl also produced progeny with no spines, predominantly, but with a few one-spined progeny. The early appearance of this mutation gave it greater prominence than it would have enjoyed later on, since ldl and three of its offspring were included among the progenitors of low lines. In all, 39 individuals can be traced back to ldl as their original ancestor. Of these, 33 had no spines and 6 had one spine. But the effect on the final result of the experiment is inconsiderable. If all of the offspring of the mutation are left out of the table, the number of individuals produced by the low lines between August 26 and September 3 is reduced to 168, with

an average spine number of 1.867. This still gives a difference between the average spine numbers of the high and low groups of .88 ± .07

The very favorable character of the race employed in experiment 4, that is, the close correspondence between its external appearance and genetic constitution, is shown by the high correlation between parent and progeny. The coefficient of correlation between parents and progeny, for spine number, is .376 ± .017 for the 1277 individuals of this race. This is the highest coefficient of correlation within a clone that has been obtained in the course of my experiments. If the mutation ldl and its offspring are omitted, the coefficient becomes .282 ± .018.

Conclusions.

Results of the present investigation. The present study of inheritance in Centropyxis may be divided into two parts, the first concerned with a statistical analysis of inheritance within the population and within single clones, the second with an attempt to test the inheritance of variations in a single definite character within the clone, by the method of selection.

The results of the biometrical study of inheritance in Centropyxis may be summarized as follows.

1. There exist in the species, Centropyxis aculeata, many diverse strains or clones which, under identical environmental conditions, differ from each other decidedly in spine number, shell size, mouth size and fission rate, but differ only slightly in shell form and mouth form.

2. In a population, the coefficients of correlation show that variations in spine number, shell size and mouth size are strongly inherited, while variations in shell form and mouth form are weakly inherited.

3. In a single clone, the coefficients of correlation show that variations in spine number and shell size are probably inherited.

4. If a diminishing ancestral correlation is considered a criterion of inheritance of variations within a clone, the coefficients of correlation of the population give such evidence of inheritance for all the characters studied, and the coefficients of correlation within a clone give such evidence for spine number and shell size.

5. From the evidence adduced under 2, 3 and 4 above, it was concluded that variations in spine number and shell size are inherited within the clone. Since shell size might be "inherited" without the mediation of the nucleus, spine number was considered the most favorable character for use in selection experiments.

The results of the experiments in the selection of variations in spine number may be summarized as follows.

6. In the brief experiments 1 and 2, mass selection based on external appearance gave contradictory results in two different races. In explanation of this, it is assumed that in different races the degree of correlation between the external appearance of the individual and its genetic constitution is diverse; in some the two are highly correlated, in others but little.

7. In experiment 3, individual selection based on the average spine number of the progeny of the individual under consideration resulted in a rapid isolation of two decidedly different strains within a single clone.
8. Since the result in experiment 3 might have been due to the appearance and preservation of a mutation, mass selection was again tried. In experiment 4, on a very favorable race (as is shown by its high parental correlation), mass selection based on the external appearance of the individual was sufficiently effective to isolate two distinct strains from within a single clone. In this experiment 1277 individuals were obtained, all coming originally from a single specimen.
9. In this experiment 4, something resembling a mutation did appear. Its 39 progeny were traced out, and it was shown that their elimination from the record makes no essential change in the final result.

Present status of the "pure line" hypothesis. In considering the bearing of this work and other similar investigations on the concept of the "pure line", one must carefully distinguish between the two parts of which this hypothesis is composed. The term "pure line" was originally applied to relatively constant diverse strains, discovered within a single species. That such relatively constant strains do occur in a great variety of plant and animal species, is an observational fact, and has been reaffirmed by every investigator who has entered this field.

The further idea, that within one of these "pure lines" no variation in genetic constitution is possible, except, perhaps, by a sudden large mutation, is not direct observation but hypothesis. It is an hypothesis, to be sure, which was based on all the experimental evidence available at the time it was formulated, but it is still an hypothesis, subject to revision at any time when new experimental results demand it.

For some years, the great majority of the experimental results tended to confirm this hypothesis, and it gained such a firm footing that all the contradictory cases were dismissed as aberrant types, results of a failure to properly control the environment, observational errors, etc. But of late the tide seems to be turning somewhat. Stout (1915), in Coleus, Stocking (1915) in Paramecium, Middleton (1915) in Stylonychia, and Jennings (1916) in Difflugia have all shown that minute heritable variations do occur within clones derived from a single ancestor by vegetative multiplication. In the present work on Centropyxis, the same conclusion was reached.

We must admit, it seems to me, that in these plants and protozoans reproducing asexually, small variations in genetic constitution do continually occur. How does this affect the "pure line" hypothesis ? The issue can be met in two ways.

In the first place, it is still possible to reaffirm the entire validity of the original pure line hypothesis.

The new conflicting results, like previous ones, can still be explained away as due to complicating factors. For example, the inheritance of abnormalities in Paramecium (Stocking 1915) can be considered to follow from an abnormal condition of the macro-nucleus; the inheritance of variations in fission-rate in Stylonychia (Middleton 1915) might be due to the accumulation of waste products in the cytoplasm of the slowly-dividing, large-sized group. And in Difflugia and Centropyxis (Jennings 1916 and the present paper), until we know more about the process of cell division in these organisms, the observed inheritance of variations can be explained by the assumption that a process of somatic segregation or fractionation of the chromidial body takes place at each cell division, as has been suggested by Morgan (1916,p.185). Other similar cases could doubtless be supplied with other explanations along the same lines.

But there is another viewpoint, alternative to the first one, which seems to me to be preferable. According to this idea, one may assert that the pure line concept is correct on the average, only; that it is a mathematically correct expression of the mean result of inheritance under natural conditions. But, just as the Galton-Pearson law of ancestral inheritance, though accurate mathematically, does not fit the details of Mendelian heredity, on the average result of which it is presumably based, so the pure line hypothesis, though true for average results, may be thought of as not holding in individual cases.

Or, to look at the question from another side, just as species were found to consist of a number of pure lines, diverse from each other in minor details of genetic constitution, so we may think of pure lines as made up of a number of individuals, differing from each other in even smaller details which are, nevertheless, hereditary.

According to this idea, we can assume that each individual inherits, in general outlines, the genetic constitution of its species; in smaller details, that of its pure line; in still more minute details, that of its individual parent; and yet may differ from this parent in some hereditary points. If these individual hereditary differences are fairly large, they may be referred to as mutations. If they are very minute, they can be termed genetic fluctuations, micro-mutations, or any other term which is found to meet the needs of those who work in this field.

So far as our present knowledge goes, either of these two views may be championed with equal success. The final decision between them must be left to the experimental results of the future.

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Appendix.

Some important correlation tables.

1. Correlation between spine numbers of parents and their progeny in the population.

	0	1	2	3	4	5	6	7	8	9	10	
0	25	33	18	2	2							80
1	26	37	30	6	3							102
2	19	41	19	4	4	1						88
3	6	12	6	3	1	2	2	1				33
4		3		2		6	15	7	1			34
5		1		5	10	33	43	33	17	3	3	148
6				8	8	51	109	49	31	13	2	271
7				8	2	28	85	41	23	6	2	195
8				3	1	18	32	15	9	2	1	81
9					1		8	8	2	1		20
10							1	1	2			4
	76	127	73	41	32	139	295	155	85	25	8	1056

2. Correlation between shell size indices of parents and their progeny in the population.

	17	19	21	23	25	27	29	31	33	35	37	39	41	43	45	
17	2	5	16	5												28
19	8	33	71	16	4	1	4									137
21	6	69	192	71	26	1	7									372
23		20	85	48	15	6	3									177
25			15	6	3	2	1		1							28
27						1	1		1							3
29						1					1	1	2			5
31									7	5	5	8	1		1	27
33							3	2	8	14	7	13	2		4	53
35								3	12	10	12	21	2	1	3	64
37							6	2	12	15	7	23	3		2	70
39							1		6	8	7	20	3		3	48
41							1		1	3	6	5		1		17
43										1	2	4				7
45										1		2				3
	16	127	379	146	48	12	27	7	48	57	47	97	13	2	13	1039

3. Correlation between shell size indices of grandparents and progeny in the population.

	17	19	21	23	25	27	29	31	33	35	37	39	41	43	45	
17		4	13	6	1	1	2									27
19	1	22	71	15	4	14	10									137
21	1	49	201	59	28	23	14									375
23		21	96	30	11	12	9									179
25		1	17	1	1	3	3									26
27							1									1
29							2				1			1		4
31							1	1	5		4	7			4	22
33							2		6	7	9	19			6	49
35							3		5	9	11	21		1	7	57
37							3	5	8	7	8	24			6	61
39							1		2	6	5	19		1	5	39
41							2		1	3	4	5			2	17
43							1	1	1			2			1	6
45								1								1
	2	97	398	111	45	53	54	8	28	32	42	97	0	3	31	1001

4. Correlation between spine numbers of parents and progeny in line 30.

	3	4	5	6	7	8	9	10	
3	1		2	2	1				6
4	2		6	15	7	1			31
5	5	10	33	43	33	17	3	3	147
6	8	8	51	109	49	31	13	2	271
7	8	2	28	85	41	23	6	2	195
8	3	1	18	32	15	9	2	1	81
9		1		8	8	2	1		20
10				1	1	2			4
	27	22	138	295	155	85	25	8	755

5. Correlation between spine numbers of grandparents and progeny in line 30.

	3	4	5	6	7	8	9	
3	1	1	1	2				5
4	6	3	6	6	6	4		31
5	7	5	21	70	33	7	3	146
6	22	11	45	119	55	10	9	271
7	16	9	39	81	37	10	4	196
8	6	3	14	36	14	6	2	81
9		1	3	10	4	1	1	20
10				3	1			4
	58	33	129	327	150	38	19	754

6. Correlation between the spine numbers of great-grand-parents and progeny in line 30.

	3	4	5	6	7	8	9	
3	3	1	1					5
4	5	1	5	14	3	1	1	30
5	26	10	27	60	20	2		145
6	39	16	52	128	26	5	2	268
7	37	11	33	85	21	3	2	192
8	14	2	17	37	6	2	2	80
9	7		2	9	2			20
10				4				4
	131	41	137	337	78	13	7	744

7. Correlation between shell size indices of parents and progeny in line 30.

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
16								1							1
17				1		3	1	1							6
18		2		2	2	3	9	1	2						21
19		2	1	6	9	11	8	2		2					41
20		3	2	8	10	22	30	10	4	2		1		4	96
21		2	1	11	25	44	53	21	9	12				3	181
22		2	1	9	24	41	53	31	10	14		1		4	190
23				2	11	21	38	23	10	7	3	4	1	3	123
24				1	6	10	16	13	2	5		1			54
25						4	8	3	1	2		2		1	21
26						1	2	1	1	1					6
27														1	1
28												1			1
29												1			1
		11	5	40	87	160	218	107	39	45	3	11	1	16	743

8. Correlation between spine numbers of parents and progeny in line 9.

	0	1	2	3	4	
0	10	11	6		1	28
1	7	8	12	2	1	30
2	5	11	8	1	1	26
3	1	2	2		1	6
4		2				2
	23	34	28	3	4	92

9. Correlation between spine numbers of parents and progeny in line 41.

	0	1	2	3	4	5	
0	9	8	8	2	1		28
1	16	14	8	2	2		42
2	12	15	7	2	3	1	40
3	5	7	3	2			17
4		1					1
5		1					1
	42	46	26	8	6	1	129

10. Correlation between spine numbers of parents and progeny in line 43.

	0	1	2	3	
0	6	14	4		24
1	3	15	10	2	30
2	2	15	4	1	22
3		3	1		4
	11	47	19	3	80

11. Correlation between spine numbers of parents and progeny during Selection experiment 4.

	0	1	2	3	4	
0	26	14				40
1	11	116	82	78	24	311
2		70	70	106	33	279
3	1	56	82	204	98	441
4		41	34	84	46	205
	38	297	268	472	201	1276

VITA.

Francis Metcalf Root was born at Oberlin, Ohio on September 24, 1889. He prepared for college at Oberlin Academy, graduating in 1907. He attended Oberlin College from 1907 to 1912, receiving the degree of A. B. in 1911, and that of M. A. in 1912. During the year 1911-12 he held a graduate scholarship in Biology. In 1912 he came to the Johns Hopkins University as graduate assistant to Dr. H. S. Jennings, a position which he held for two years. In this University, his major subject was Zoology and his minors were Plant Physiology and Botany. He held a University Fellowship in Zoology during the year 1914-15 and held the Adam T. Bruce Fellowship during the years 1915-16 and 1916-17.